Brit. J. vener. Dis. (1972) **48**, 97

Fluorescent treponemal antibody-cerebrospinal fluid (FTA-CSF) test

A provisional technique

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Results of modification of the fluorescent treponemal antibody (FTA) test (Harris, Bossak, Deacon, and Bunch, 1960), using sequentially collected cerebrospinal fluid (CSF) from four chimpanzees infected with *Treponema pallidum*, were recently reported (Duncan and Kuhn, 1972). The results suggested that a procedure be evaluated using undiluted CSF collected from humans infected with *T. pallidum*.

In the chimpanzee study, the time of infection was documented, specific treatment was withheld, and other experimental conditions were well controlled. In the present study, one CSF specimen was obtained from each patient. The diagnosis of each case was furnished by the physician contributing the specimen.

The results obtained when standard and experimental serological tests for syphilis were performed on CSF from humans are presented and a provisional technique is proposed as an aid in the diagnosis of asymptomatic central nervous system (CNS) syphilis.

Materials and methods

(1) Human CSF samples were collected at Saint Elizabeth's Hospital, Washington, D.C.; Vanderbilt University School of Medicine, Nashville, Tennessee; Passavant Memorial Hospital, Chicago, Illinois; Henrietta Egleston Hospital for Children, Fulton County Health Department Clinic, and by Venereal Disease Branch staff, Center for Disease Control, Atlanta, Georgia. Based on the diagnosis furnished by the submitting physician at the source, the CSF specimens were placed in the categories listed below:

Received for publication September 10, 1971
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Trade names are used for identification only and do not represent an endorsement by the Public Health Service or the U.S. Department of Health, Education, and Welfare

Category		*No. of specimens*
Nonsyphilitic		31
Syphilitic	Primary	40
	Secondary	27
	Early latent	54
	Late latent	172
	Cardiovascular	4
	Congenital	26
	Central nervous system	38
Total		392

Of the 392 CSF samples, 335 were obtained from a specimen bank maintained at the Venereal Disease Research Laboratory (VDRL), Center for Disease Control, Atlanta, Georgia. These specimens had been divided into 1-ml. amounts, placed in screw-capped vials, sealed with tape, and stored at -20°C. or below. Of the remaining 57, thirteen were obtained from individuals who were part of another study on the evaluation of treatment for early syphilis (Schroeter, Lucas, Price, and Falcone, 1972). Five specimens designated as primary syphilis and eight as secondary syphilis came from patients who had been treated for their early syphilis 2 years before the CSF sample was obtained. The categorization of the bank specimens preceded the testing of the spinal fluids in the FTA modifications and the categorization of the remaining specimens preceded the FTA and VDRL slide testing.

(2) A corresponding serum specimen was obtained from individuals in the nonsyphilitic control group.

(3) The VDRL slide test with serum and spinal fluid and the fluorescent treponemal antibody-absorption (FTA-ABS) test with serum were performed by personnel of the Testing Subunit of the VDRL, according to current methods (Manual of Tests for Syphilis, 1969).

(4) Details of the testing and reporting of the FTA test with undiluted CSF (hereafter referred to as the FTA-CSF test) and with CSF diluted 1 : 5 in sorbent have

been described previously (Duncan and Kuhn, 1972). In brief, undiluted CSF or CSF diluted 1 : 5 with sorbent is overlaid on *T. pallidum* antigen affixed to microscope slides. The attachment of CSF antibodies with the treponeme is revealed by staining with a fluorescein-conjugated antiserum to human globulin, as in the FTA-ABS test on serum. A reactive report is given when the degree of fluorescence is equal to or greater than that of the 1+ reading standard, and a nonreactive report is issued when the fluorescence is below the 1+ reading standard or is absent. The Reagents Subunit of the VDRL supplied the reagents and control sera necessary for the fluorescent antibody (FA) procedures.

Results

Reactive results of the VDRL slide and the FA tests on CSF are shown in Table I. In the nonsyphilitic control group, sera from thirty of the 31 individuals were nonreactive in the FTA–ABS test, and one was borderline. Corresponding CSF from this control group were completely nonreactive in both the VDRL slide test and the FTA–CSF test. Of the specimens from the syphilis categories, fourteen were reactive with the VDRL slide spinal fluid test and 82 with the FTA–CSF test.

Of the fourteen specimens reactive in the VDRL slide test, thirteen were from the CNS syphilis category and one was from a patient who was taking part in a study on the evaluation of treatment for early syphilis. Upon return of the VDRL slide test report to the submitting source, the diagnosis of this one case was changed to asymptomatic CNS syphilis. Thus, all fourteen reactive results were obtained on specimens from individuals who had a diagnosis of CNS syphilis. However, 25 specimens of the CNS syphilis category failed to react with the VDRL slide test.

Of the 82 specimens reactive with the FTA–CSF test, 78 specimens were in the latent and CNS syphilis categories, two were in the congenital category, and one each in the primary and secondary syphilis categories. The one specimen in the primary category gave the reactive VDRL slide test mentioned above.

The results of the FA test with CSF diluted 1 : 5 in sorbent, which is analogous to the FTA–ABS serum test, showed that only 37 spinal fluids remained reactive; eighteen were in the latent category and nineteen in the CNS syphilis category. One specimen from the CNS category was nonreactive with the 1 : 5 sorbent dilution but reactive with the VDRL slide test.

At the completion of this study the available clinical history forms of patients in the CNS syphilis category were reviewed and the information obtained is shown in Table II (opposite).

Results of nontreponemal tests performed on serum at the submitting source showed that 23 were reactive, five weakly reactive, eight nonreactive, and two were not available. Test results on CSF showed fourteen were reactive, eighteen were nonreactive, and six were not available. The white cell counts were greater than four per cu. mm. in thirteen specimens, four or less in twelve specimens, 0 in eight, and five counts were not available. The protein content was elevated above 40 mg. per cent. in 28 of the spinal fluids. Eighteen individual records had notations of clinical manifestations consistent with the diagnosis of CNS syphilis, sixteen were listed as asymptomatic, and no notations were given for four individuals.

Discussion

Although it is said that the incidence of neurosyphilis is declining, the continuing clinical importance of the late complications is attested to by the number of papers published over the last few years

TABLE I *Summary of reactive test results*

Category		*Number of tests*	*VDRL test*	*FTA test*	
			CSF undiluted	*CSF undiluted*	*CSF diluted 1 : 5 in sorbent*
Nonsyphilitic		31			
Syphilitic	Primary	40	1*	1*	
	Secondary	27		1	
	Early latent	54		9	
	Late latent	172		38	18
	Cardiovascular	4			
	Congenital	26		2	
	Central nervous system	38	13	31	19
Total		392	14	82	37
Per cent. reactive		—	3·6	20·9	9·4

*Diagnosis changed to asymptomatic neurosyphilis after the VDRL slide test was reported

TABLE II *Spinal fluid test results and information from clinical histories of 38 patients with CNS syphilis*

Patient No.	*Test results*		*Clinical history information*					
			Laboratory results				*Physicians notations*	
	VDRL slide test	*FTA-CSF test*	*Serological test*		*Cells (per mm.³)*	*Total protein (mg. per cent.)*	*Previous treatment*	*Clinical impression*
			Serum	*CSF*				
1	R	R	R	R	6	60	I	Paresis
2	NR	R	WR	NR	0	40	A	Asymptomatic
3	R	R	R	R	6	96	None	Optic atrophy
4	R	R	R	R	15	117	I	Paresis
5	NR	R			0	41	—	—
6	NR	WR	WR	NR	0	49	A	Meningovascular
7	NR	R	R	R	1	40	I	Asymptomatic
8	NR	R	R	NR	2	36	A	Asymptomatic
9	R	R	R			54	—	—
10	R	R	R	R	2	43	I	Meningovascular
11	R	R	NR	R	46	52	I	Tabes dorsalis
12	R	R					A	Meningovascular
13	NR	NR	R	NR	0	43	None	Optic atrophy
14	NR	R	R	NR	0	43	None	Tabes dorsalis Argyll Robertson pupils
15	NR	R	R	NR	1	37	A	Asymptomatic
16	NR	R	R				—	—
17	NR	R	NR	NR	0	43	A	Optic atrophy
18	R	R	R	R	7	64	None	Paresis
19	NR	NR	NR	NR	2	46	A	Asymptomatic
20	NR	R	R				—	—
21	NR	R	R	R	15	40	None	Chorioretinitis
22	NR	NR	NR	NR	3	76	A	Asymptomatic
23	NR	R	WR	NR	3	35	A	Asymptomatic
24	R	R	R	R	5	58	I	Asymptomatic
25	NR	NR	NR	NR	1	43	A	Asymptomatic
26	NR	R	R	R	1	46	A	Pupillary changes
27	NR	R	R				—	—
28	NR	R	WR	NR	4	46	A	Asymptomatic
29	R	R	R	R	6	55	I	Asymptomatic
30	NR	NR	R	NR	1	52	I	Chorioretinitis
31	NR	R	R	NR	0	43	A	Asymptomatic
32	R	R	R	R	0	58	None	Chorioretinitis
33	NR	NR	WR	NR	13	49	A	Asymptomatic
34	NR	R	NR	NR	5	49	A	Asymptomatic Gonorrhoea
35	R	R	R	R	73	46	None	Meningovascular
36	NR	NR	NR	NR	22	67	I	Asymptomatic
37	NR	R	NR	NR	1	44	A	Asymptomatic
38	R	R	R	R	30	67	—	Paresis

NR = Nonreactive WR = Weakly reactive R = Reactive I = Inadequate treatment A = Adequate treatment

(Harner, Smith, and Israel, 1968; Dewhurst, 1968; Ley and Kridde, 1968; Joffe, Black, and Floyd, 1968; Dewhurst, 1969; Förström and Lassus, 1969; Aho, Sievers, and Salo, 1969; Starzycki, Mayer, Huczynski, and Rataj, 1970). Dewhurst (1969) reported on a survey of 91 cases. He emphasized the necessity of early diagnosis and treatment, and suggested that, if there is a clinical possibility of neurosyphilis, the spinal fluid should be examined even though the blood tests are nonreactive. An example of this type of situation, a nonreactive serological test on serum with a reactive result using spinal fluid, was demonstrated with Patients 11, 17, 34, and 37 (Table II) in this study. Starzycki and others (1970) reported that 34·3 per cent. of patients with tabes dorsalis had nonreactive blood tests. In this series, 23·9 per cent. of the patients with nonreactive serum tests had reactive spinal fluid test results. Joffe and others (1968) have suggested that diagnosis of neurosyphilis may be complicated by the use of treponemicidal drugs for conditions other than syphilis.

Since all neurosyphilis is asymptomatic at some time during the course of infection (US Dept. HEW, 1968), a sensitive and specific test to detect the early CSF changes that occur after CNS invasion with *T. pallidum* is desirable. It is now generally agreed that the FTA–ABS serum test is the most sensitive of any treponemal or nontreponemal test used in syphilis serology (Moore and Knox, 1965; Smith, 1967; Mackey, Price, Knox, and Scotti, 1969). The test is

one modification of the original fluorescent test for treponemal antibodies, published by Deacon, Falcone and Harris (1957).

The first FTA test with human spinal fluids was reported by Harris and others (1960); it was performed in a manner similar to the procedure with undiluted CSF used in this study but had the addition of rotating slides during the incubation periods and a different criterion for reporting results. The authors found the FTA technique to be more sensitive than the VDRL slide, the *Treponema pallidum* immobilization-200 (TPI-200), and the Kolmer Reiter protein antigen (KRP) tests. However, they could not decide if this greater sensitivity provided clinically useful information.

The use of the FTA, FTA–ABS, and other related FA tests with CSF was reported by a number of investigators (Vaisman and Hamelin, 1961; Neil and Fribourg-Blanc, 1964; Ripault and Colombani, 1964; Ruczkowska, 1965; Naumann, 1965; Camargo and Bittencourt, 1966; Johnston and Wilkinson, 1968; Lassus, 1968; Förström and Lassus, 1969; Aho and others, 1969; Datta and Mitra, 1969; Dyachenko, 1969; Escobar, Dalton, and Allison, 1970). All studies have indicated that the FA tests with CSF were more sensitive than any other tests with which they had been compared, and specificity of the tests was also claimed.

Even though the original FTA test on CSF specimens was shown to have the greatest sensitivity, the technique did not come into general use. The feeling then, and as late as 1967 (US Dept. HEW, 1968) was that a treponemal type test was not necessary to provide specificity in the testing of CSF, since false positive nontreponemal test reactions in the CSF are rare. However, the desirability of additional sensitivity as afforded by the FTA procedure does not yet seem to have been decided. Predominant clinical opinion holds that active neurosyphilis will declare itself by a reactive VDRL slide test in the CSF. Thus, by this reasoning, CSF which has only a reactive FTA–CSF test, is not clinically important, although it is immunologically interesting. Clearly, additional clinical studies are needed to assess the clinical relevance of the greater sensitivity offered by the FTA–CSF procedure and it is hoped this report will help stimulate further evaluations.

This present study confirms and extends the previous work in showing that the VDRL slide test, which is probably the most widely used spinal fluid test, is less sensitive than the FA methods used. Reactive FTA–CSF results were obtained in all syphilis categories except that of the cardiovascular group; however, only four specimens were in this category.

Merritt (1967) stated that 'The central nervous system is invaded by the *Treponema pallidum* within a few weeks or months of the original infection. Abnormalities are present in the cerebrospinal fluid in a small percentage of cases in the primary stage of the disease and in over one-third of cases in the secondary stage'. Surprisingly, in the light of these statements, only two reactive FTA–CSF results were obtained among the 67 specimens in the early syphilis categories.

This and other studies (Harris and others, 1960; Escobar and others, 1970; Duncan and Kuhn, 1972) showed that the reactivity of the CSF decreased when diluted in either saline or sorbent, and that the reactivity decreased inversely with the dilution factor. This argues for the use of undiluted CSF in the FTA–CSF test. However, reactivity of the FTA–CSF test, as with other treponemal tests, need not necessarily indicate an active disease process.

An interesting observation in support of the sensitivity of the FA test was made by Mattern, Pillot, and Bernardot (1965). Using immunofluorescence and immunoelectrophoretic techniques, they found an increase in the IgG titre in spinal fluid associated with neurosyphilis and observed the appearance of IgA and IgM. They concluded that the IgG antibody found in the spinal fluid was elaborated in the fluid itself and had more antitreponemal properties than serum. This may be the cause of the greater reactivity of the FTA–CSF test in which *T. pallidum* is used as antigen compared to the lesser reactivity of the nontreponemal VDRL slide test with its cardiolipin antigen.

Because of the decrease in reactivity when CSF was diluted in sorbent, it is recommended that no sorbent be used and that, for maximum sensitivity, undiluted unheated CSF be used in the FTA–CSF test. The other procedural steps of the present FTA–ABS test ('Manual of Tests for Syphilis', 1969), *i.e.* time of incubation, rinsing, use of controls, etc., should be followed. However, at this time, we suggest that the results of the FTA–CSF test be reported as reactive or nonreactive, until additional correlation of results with the clinical manifestations can be made.

The specificity of the FTA–CSF test appears to be adequate, but it is stressed that only 31 nonsyphilitic CSF specimens were examined. Obviously, more nonsyphilitic samples are needed in future evaluations before it can be safely concluded that the greater sensitivity of the FTA–CSF test is not accomplished at the expense of specificity.

Summary

Cerebrospinal fluid (CSF) from syphilitic patients

and nonsyphilitic subjects was examined with the VDRL slide test and with two immunofluorescence procedures; one using undiluted CSF (FTA–CSF test) and one using CSF diluted 1 : 5 with sorbent. The FTA–CSF test was the most reactive and the VDRL test was the least reactive. CSF diluted 1 : 5 in sorbent was less reactive than undiluted CSF.

The FTA–CSF procedure is proposed as a provisional technique for a sensitive CSF test. The current FTA–ABS technique is followed, except that (1) the CSF is not heated; (2) it is not diluted with sorbent; and (3) the report of the test should be limited to reactive or nonreactive results. The FTA–CSF provisional technique requires further clinical and laboratory evaluation by others, in regard both to technical aspects and to the clinical value of increased detection of *T. pallidum* antibody in the CSF. Further nonsyphilitic patients must be studied to determine if false positive reactions ever occur. If subsequent studies confirm the findings presented here, a standard technique for immunofluorescence testing of CSF could be recommended.

We wish to thank Miss Genevieve W. Stout for encouragement and assistance, and her staff for the results of the VDRL slide and FTA–ABS tests. We also thank Drs. Bobby C. Brown, Rudolph H. Kampmeier, Herbert M. Sommers, and Richard J. O'Reilly, for obtaining spinal fluid specimens used in the study.

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Le test des anticorps tréponémiques fluorescents dans le liquide céphalo-rachidien (FTA-CSF): technique provisoire

SOMMAIRE

Le liquide céphalo-rachidien (LCR) de syphilitiques et de sujets non syphilitiques fut examiné au VDRL sur lame et avec deux techniques d'immunofluorescence: l'une sur LCR non dilué (test FTA–CSF) et l'autre sur LCR dilué au 1/5ème dans un liquide de sorption. Le test le plus réactif fut le FTA–CSF, et le moins réactif fut le VDRL. Le LCR dilué au 1/5ème fut moins réactif que le LCR non dilué.

Le FTA–CSF est proposé comme technique provisoire d'un test sensible pour le LCR. C'est la technique habituelle du FTA–ABS qui est employée, sauf que (1) le LCR n'est pas chauffé; (2) qu'il n'est pas dilué dans un liquide de sorption; et (3) que les réponses du test doivent se contenter d'indiquer positif ou négatif. La technique provisoire FTA–CSF demande des suppléments d'appreciation par d'autres, en clinique et au laboratoire, aussi bien sur le plan technique que sur la valeur clinique d'une meilleure détection de l'anticorps tréponémique dans le LCR. Des études supplémentaires doivent être faites chez des malades non syphilitiques pour établir s'il peut exister des réactions faussement positives. Si ces études ultérieures confirment les présentes recherches, une technique standard pour l'examen du LCR en immunofluorescence pourra être recommandée.